Nutritional Evaluation of Milk Processed for Removal of Cationic Radionuclides. Feeding Studies

R. E. ISAACKS,[1,3] D. G. HAZZARD,[1] JULIUS BARTH,[1] J. P. WALKER,[1] J. H. FOOKS,[1] AND L. F. EDMONDSON[2]

One experiment involving 84 rats and two experiments involving 60 baby pigs were conducted to compare the relative nutritive value of ion exchange–processed whole milk powder (treated) with control whole milk powder (untreated). The experiments measured the rate of weight gain, blood serum cation concentrations, and urine cation excretions. There was no significant difference ($P > 0.05$) in rate of growth or in blood serum cations, in either the rats or baby pigs, between the groups of animals fed processed whole milk or fed the control whole milk. There was significantly ($P < 0.05$) greater urine potassium excretion from pigs fed processed milk or control milk plus potassium citrate as compared with pigs fed the control whole milk. One experiment showed a significantly ($P < 0.05$) greater excretion of sodium from pigs fed control whole milk plus potassium citrate as compared with pigs fed control whole milk.

Chemical analyses on fluid whole milk processed for removal of cationic radionuclides by ion exchange techniques showed that the potassium content and per cent ash were increased by 80 and 14%, respectively, while thiamine, niacin, and vitamin B_6 content were decreased by 50, 27, and 15%, respectively, compared with the control milk (9). The objective of the studies was to determine the effect of the alterations in the milk composition as a result of the ion exchange process. Whole milk powder of control milk (untreated) and processed milk (treated) was fed to both rats and baby pigs. The rates of growth were observed and the levels of the major cations, particularly potassium, were determined in the serum and urine of these species.

Assessment of the nutritive qualities of the milk by feeding studies tests not only the severity of the observed chemical alterations (9), but also tests for possible alterations in milk composition as a result of the spray-drying process described previously (9).

Experimental

The dried whole milk powder for both the rat feeding study and the two baby pig feeding studies was prepared as described earlier (9).

Rat Study. The animals used in this experiment were male and female albino rats from a colony descended primarily from the Wistar strain, but maintained as a separate unit at Beltsville, Md., for more than 20 years. The young rats were weaned at 25 days of age and started on experiment (Table I) at about 28 days of age. Six male and six female litter mates were randomly assigned to each of six groups and each group was replicated seven times for a total of 84 rats on experiment. During the experimental period, the rats were maintained in individual cages provided with raised screen floors. Rations (Table II) and distilled water were supplied *ad libitum*. A mineral supplement containing iron, copper, and manganese was added to groups 1 and 2. The rats were individually weighed initially and at weekly intervals for 12 weeks. Records of individual feed consumption and water consumption were kept. After 12 weeks on experiment, the rats were placed in individual metabolism cages for a 24-hour urine collection, then sacrificed. Immediately preceding sacrifice, blood samples were taken by heart puncture for subsequent analyses. Blood serum sodium and potassium and urine sodium and potassium were determined by flame photometry (2), and serum calcium and magnesium were determined by a modified titrimetric method of Todd (15). In the modified procedure, Cal-red indicator was used for calcium titration, and Eriochrome Black T indicator was used for total (calcium plus magnesium) titration, magnesium being calculated by difference. Todd (15) used Eriochrome Black T indicator for both magnesium titration and total (magnesium plus calcium) titration, calcium being calculated by difference. Calcium in the urine was determined by flame photometry (3).

Baby Pig Experiment 1. The animals used in this experiment (Table III) were 3-day-old cross-bred piglets weighing from 1.37 to 2.05 kg. The piglets were divided into three treatment groups with each group comprised of two lots (replicates) of six pigs each for a total of 36 pigs. Litter mates of each sex were distributed at random between the six lots. Each lot of six pigs was housed in a raised screen floor pen under heat lamps. A mineral supplement containing iron, copper, manganese, and zinc was added to the three treatment diets (Table III). The whole milk powders were reconstituted (12.5% solids) and

Table I. Experimental Design for Weanling Rat Study

Group	Treatment
1	Control milk + (Fe, Cu, Mn)[a]
2	Processed milk + (Fe, Cu, Mn)[a]
3	Diets I[b] + control milk
4	Diets I[b] + processed milk
5	Diets II[b] + control milk
6	Diets II[b] + processed milk

[a] Trace mineral mixture supplement supplied per kilogram of dried whole milk powder: 40 mg. copper (157.06 mg. $CuSO_4 \cdot 5H_2O$), 80 mg. iron (398.34 mg. $FeSO_4 \cdot 7H_2O$), 40 mg. manganese (162.39 mg. $MnSO_4 \cdot 4H_2O$).
[b] See Table II.

Table II. Experimental Diets for Weanling Rat Study

Ingredients	Diet I, %	Diet II, %
Casein (vitamin-free)	13.10	13.10
Dried whole milk (either control or processed)	50.00	50.00
Cerelose (glucose monohydrate)	31.55	29.30
Wood pulp	3.00	3.00
Choline chloride	0.10	0.10
Vitamin mixture[a]	1.00	2.00
Mineral mixture[b]	1.25	2.50
	100.00	100.00

[a] Vitamin mixture supplement supplied per kilogram of diet: 1.0 mg. thiamine hydrochloride, 1.5 mg. riboflavin, 1.5 mg. pyridoxine hydrochloride, 10.0 mg. calcium pantothenate, 0.2 mg. folic acid, 20.0 mg. nicotinic acid, 20.0 mg. inositol, 120.0 mg. p-aminobenzoic acid, 0.05 mg. vitamin B_{12}, 750 mg. menadione, 0.10 mg. biotin, 2000.0 mg. DL-methionine, 2.0 mg. alpha-tocopheryl acetate, 30,000 I.U. vitamin A, and 4000 I.U. vitamin D in 7.816 grams of cerelose as carrier.
[b] Mineral mixture supplement supplied per kilogram of diet: 2.67 grams $CaCO_3$, 4.16 grams $CaHPO_4 \cdot 2H_2O$, 0.25 gram $MgCO_3$, 0.30 gram $MgSO_4 \cdot 7H_2O$, 1.27 grams NaCl, 0.21 gram KCl, 3.15 grams KH_2PO_4, 0.20 gram $FeSO_4 \cdot 7H_2O$, 0.25 gram $MnSO_4 \cdot 4H_2O$, 0.006 gram KI, 0.016 gram $CuSO_4 \cdot 5H_2O$, 0.010 gram NaF, 0.0024 gram $K_2Al_2(SO_4)_4 \cdot 24H_2O$, 0.005 gram $ZnSO_4 \cdot 7H_2O$, and 0.0006 gram $CoCl_2 \cdot 6H_2O$.

Table III. Experimental Design for Baby Pig Studies

Group	Treatment
1	Control milk powder + (Fe, Cu, Mn, Zn)[a]
2	Processed milk powder + (Fe, Cu, Mn, Zn)[a]
3	Control milk powder + (Fe, Cu, Mn, Zn)[a] + potassium citrate[b]

[a] Trace mineral mixture supplement supplied per kilogram of milk powder: 117.9 mg. iron (0.5896 gram $FeSO_4 \cdot H_2O$), 3.1 mg. copper (0.0121 gram $CuSO_4 \cdot 5H_2O$), 21.7 mg. manganese (0.0598 gram $MnSO_4 \cdot 4H_2O$), 50.5 mg. zinc (0.0629 gram ZnO), and 20.0 mg. oxytetracycline.
[b] Potassium citrate added at level of 29.38 grams per kilogram of milk powder, equivalent to level of potassium and citric acid added during process.

placed in an 8-quart, round, milk kettle ($8\frac{1}{2}$ inches in diameter and $18\frac{1}{2}$ inches high on a 22-inch base; Pigsaver, Norwood Products Co., Luverne, Minn.) with eight anticolic nipples evenly spaced around each kettle. The milk was reconstituted twice daily, and each lot of six piglets was fed on a basis of "grams of milk powder per piglet per feeding." After the piglets had learned to nurse, the milk solids were gradually

increased to the average milk solids content of sow's milk (19%). Between 3 and 4 weeks of age, the milk solids were gradually increased to 28 to 30%. The piglets were weighed initially and at 1, 2, 4, and 8 weeks of age. At 8 weeks, the piglets were placed in large rabbit metabolism cages, and urine was collected for 24 hours. Blood samples were taken by vena cava puncture for subsequent chemical analyses. Blood serum sodium, potassium, calcium, and magnesium and urine sodium, potassium, and calcium were determined by the procedures used in the rat study.

Baby Pig Experiment 2. The animals used in this experiment (Table III) were also 3-day-old cross-bred piglets weighing from 1.10 to 1.90 kg. Each of the three treatment groups consisted of two lots of four pigs each for a total of 24 piglets on experiment. In this study, the six pens used in the first study were quartered into individual raised screen floor pens with individual heat lamps. Litter mates of each sex were again distributed at random among the six lots. The milk powders were reconstituted and fed in a manner similar to experiment 1, except that all pigs were individually fed and all pigs received control milk for one week prior to initiation of experimental treatments (Table III). The study was terminated after 4 weeks on experimental treatments. At termination, urine collections and blood samples were taken and analyzed in the same manner as in pig experiment 1.

Results and Discussion

Milk has often been termed the universal food because of its nutritious qualities and its universal usage and availability. However, it was shown some years ago by Waddell, Steenbock, and Hart (16) that bovine fluid whole milk alone would not support normal growth of rats. The same workers observed that the anemias accompanying poor growth from feeding bovine whole milk were cured or prevented with 0.5 mg. of copper and 1.0 mg. of iron per 100 cc. of fluid whole milk. Later observation showed that 0.5 mg. of manganese per 100 cc. of fluid whole milk was essential for normal reproduction and prevention of testicular degeneration. These workers also observed that fluid whole milk would not supply the energy needs of rats after 7 or 8 weeks of age, as indicated by delayed sexual maturity. Based upon these earlier findings, the control (untreated) and processed (treated) fluid whole milks were spray-dried to support normal growth better by increasing the energy density of the diets. Copper (0.5 mg. per 100 cc.), iron (1.0 mg. per 100 cc.), and manganese (0.5 mg. per 100 cc.) were added to the whole milk powders, based upon 12.5% total solids, at the level of 40 mg. of copper, 80 mg. of iron, and 40 mg. of manganese per kg. (Table I).

The vitamin levels used in diet I (groups 3 and 4, Table I) were approximately $\frac{1}{10}$ to $\frac{1}{5}$ the vitamin levels used normally in rat diets for this particular colony (6). The vitamin levels used in diet II were twice the levels of diet I.

The mineral levels used in diet I were $\frac{1}{4}$ the level used normally in experimental rat rations (12), whereas the

mineral levels used in diet II were twice the levels of diet I. The purpose of such dietary regimes was to stress the animals with suboptimum (groups 1 and 2), marginal (groups 3 and 4), and optimum (groups 5 and 6) levels of both vitamins and minerals.

Rats lose weight when fed human milk, but make satisfactory weight gains when fed bovine milk (13). Diaz et al. (5) reported that the weanling and the adult rat had a low tolerance for both lactose and galactose as measured by growth rate and urinary excretion patterns. This could account for the poor performance on human milk, since it contains approximately 57% lactose on a dry matter basis as compared to about 9% in rats' milk and about 38% in bovine milk. On the other hand, it has been demonstrated that the baby pig utilizes lactose as the carbohydrate of choice up to 3 weeks of age (5). Consequently, the baby pig has been suggested as an ideal assay animal for evaluating human infant formulas (5) and is the basis for the use of the baby pig in these studies.

Because bovine milk is known to be a poor source of many of the trace minerals, and since Diaz et al. (5) and Filer, Baur, and Rezabek (8) found it necessary to supplement whole milk formulas fed to baby pigs with a trace mineral mixture to prevent anemias and parakeratosis, the trace mineral mixture used by Filer, Baur, and Rezabek (8) was added to all the whole milk powders in these baby pig studies (Table III). Oxytetracycline (20 mg. per kg.) was added also to all diets to prevent intestinal disturbances common to baby pigs. In addition to the trace mineral mixture added to the whole milk powder, potassium citrate (29.38 grams per kg.) was added to the untreated milk (group 3, Table III), which was the calculated equivalent of the potassium (42 ml. of $0.75M$ KOH per liter of whole milk) and citric acid (16 ml. of $0.75M$ citric acid per liter of whole milk) added during the resin treatment process (7). The purpose of group 3 was to test the metabolic and renal capacity of the baby pig for this level of potassium citrate, uncomplicated by any other known or unknown alterations in whole milk as a result of the ion exchange treatment. Normally, the kidneys are very efficient in the excretion of excess water and maintenance of body fluid concentrations; however, the maintenance of water equilibrium in the infant is less efficient than in the adult (17). There seems to be little danger of potassium intoxication from careful oral and parenteral administration of potassium so long as kidney ac-

tion is efficient, but when kidney action is deficient, as in the case of diabetic acidosis and infantile diarrhea (acidosis), this danger is always present upon such administration (18). The renal capacity of animals, particularly the young, is not the only factor in considering the metabolism of the increased potassium which resulted from the ion exchange process. The excess potassium intake could cause excess sodium excretion (19), thereby affecting the entire electrolyte balance of the animal.

There is also an interrelationship between blood calcium levels and blood citric acid levels. However, Canary and Kyle (1) suggested that the citrate-calcium relationship in serum is of an indirect nature, and the varying levels of citric acid in serum in certain skeletal disorders are due to alterations in citrate hemeostasis and not to associated changes in calcium concentration of serum. The data of Schubert and Lindenbaum (14) and Neuman and Neuman (11) have shown that of the organic chelates that normally occur in blood, the citrate ion is the most potent calcium-complexing agent. However, citrate is unlikely to play a significant role in binding calcium in plasma of body fluids in vivo, as the molar concentration of bound calcium in plasma is nearly ten times higher than that of citrate, and even if all of the citrate were saturated with calcium, it would account for only one ninth of the total bound calcium (4). The considerations of the effect of added citrate to the whole milk powders as a result of the resin treatment process must also include the fact that citrate is a known metabolite of the tricarboxylic acid cycle and is metabolized very rapidly by the kidneys.

The data collected on the growth rate of rats fed the control (untreated) and processed (treated) powdered milks (group 1 vs. 2, group 3 vs. 4, and group 5 vs. 6) indicated no significant difference between these group comparisons for either males or females (Table IV). The data collected on feed and water consumption of the rats fed the control and processed milks showed no significant difference in feed consumption between group 1 vs. 2, group 3 vs. 4, or group 5 vs. 6 (Table IV). When these same comparisons were made for water consumption there was a significant difference ($P < 0.01$; P means probability) in water consumption between groups 5 and 6. The reason for the increased consumption of water from rats fed a control milk powder is unknown (Table IV). There was no significant difference ($P \geq 0.05$) between blood serum sodium, potas-

Table IV. Rat Body Weights[a] and Food and Water Consumption[b] of Study

Group	Treatment	Body Weights by Weeks				Food	Water
		0	4	8	12		
1	Control milk + TM[c]	56	173	242	270	894	2394
2	Processed milk + TM[c]	61	178	234	270	867	2236
3	Control milk, diet I	56	190	252	292	1037	2130
4	Processed milk, diet I	56	192	263	302	1088	2076
5	Control milk, diet II	57	191	254	287	1093	2642
6	Processed milk, diet II	57	184	238	281	1106	2365

[a] Average weights of male and female, grams.
[b] Average individual food and water consumption, grams.
[c] Trace mineral supplement.

sium, calcium, or magnesium concentrations in rats fed the processed milk powders (groups 2, 4, and 6) compared with the same analyses from rats fed the control milk powders (groups 1, 3, and 5; Table V). Likewise, there was no significant difference ($P \geq 0.05$) between urine sodium, potassium, or calcium values for 24-hour excretion in rats fed the processed milk powders compared with the same analyses from rats fed the control milk powders (Table V). No significant difference ($P \geq 0.05$) was found in body weight gains among the three experimental treatments in either experiment 1 or 2 (Table VI).

There was no significant difference ($P \geq 0.05$) in the blood serum concentrations of sodium, potassium, calcium, or magnesium among the three experimental treatments (Table VII). Variance in chemical procedure masked the apparent difference in serum magnesium levels in the three treatments (Table VII). In experiment 1, there was a significantly greater ($P < 0.05$) excretion of both urine potassium and urine sodium from piglets fed control milk plus potassium citrate (group 3), compared with the excretion of urine potassium and sodium from pigs fed control milk (group 1, Table VII). In experiment 2, there was again a significantly greater ($P < 0.01$) excretion of urine potassium from pigs in both groups 2 and 3 when compared with group 1 (Table VII), but urine sodium excretion was not affected.

Based upon earlier work (19) on potassium metabolism, the greater excretion of potassium in the urine as a result of greater potassium dietary levels is not surprising. However, the baby pig, as an experimental animal, is not only a more sensitive animal indicator of excess potassium intake than the rat, but the baby pig, as well as the rat, has the renal capacity to metabolize the levels of potassium and citric acid added to milk during the resin treatment process without disturbing rate of growth. Pathological observations on four pigs sacrificed from each of the three groups at 5 weeks of age indicated no abnormal microscopic findings in the kidneys, livers, or hearts of the animals (experiment 2).

The reduction of thiamine (50%), niacin (27%), and vitamin B_6 (15%) in the processed milk showed no detrimental effects in any parameters measured when compared with the pigs fed control milk powder. Calculations per kilogram of processed milk powder, from

Table V. Rat Serum and Urine Analyses at 12 Weeks

Group	Treatment	Na	K	Ca	Mg
		Serum Analyses, Mg. per 100 Ml.			
1	Control milk + TM[a]	384	13.2	16.3	0.81
2	Processed milk + TM[a]	391	13.0	15.8	0.80
3	Control milk, diet I	385	13.7	17.2	0.34
4	Processed milk, diet I	386	12.4	17.4	0.48
5	Control milk, diet II	396	11.2	18.0	0.26
6	Processed milk, diet II	391	11.8	17.8	0.34
		Urine Analyses, Mg. per 24 Hours			
1	Control milk + TM[a]	2.8	286	0.19	
2	Processed milk + TM[a]	3.6	485	0.24	
3	Control milk, diet I	3.2	246	0.18	
4	Processed milk, diet I	2.2	334	0.20	
5	Control milk, diet II	4.5	270	0.33	
6	Processed milk, diet II	3.4	390	0.22	

[a] Trace mineral supplement.

Table VI. Weight Gain of Pigs

Group	Treatment	Expt. No.	4 wk. (Kilograms)	8 wk. (Kilograms)
1	Control milk + TM[a]	1	3.30	13.04
		2	2.31	...
2	Processed milk + TM[a]	1	2.59	12.28
		2	2.83	...
3	Control milk + TM[a] + K-citrate	1	2.81	11.97
		2	2.40	...

[a] Trace mineral supplement.

Table VII. Pig Serum and Urine Analyses

Group	Treatment	Expt. No.	Na 4 wk.	Na 8 wk.	K 4 wk.	K 8 wk.	Ca 4 wk.	Ca 8 wk.	Mg 4 wk.	Mg 8 wk.
			Serum Analyses, Mg. per 100 Ml.							
1	Control milk + TM[a]	1		400		12.5		13.7		0.18
		2	348		26.0		10.7		1.74	
2	Processed milk + TM[a]	1		379		12.4		13.2		0.21
		2	343		26.8		10.6		1.49	
3	Control milk + TM[a] + K-citrate	1		394		12.4		13.1		0.20
		2	347		25.3		10.8		0.57	
			Urine Analyses, Mg. per 24 Hr.							
1	Control milk + TM[a]	1		261		2930		19.9		
		2	157		952		...			
2	Processed milk + TM[a]	1		415		3558		19.0		
		2	198		1610		...			
3	Control milk + TM[a] + K-citrate	1		776		5550		28.1		
		2	202		2268		...			

[a] Trace mineral supplement.

the fluid whole milk, based upon chemical determinations (9) indicate that thiamine, niacin, and vitamin B_6 are still present at the level of 1.12, 4.88, and 2.64 mg., respectively. The assumption was made that the manufacturing of the whole milk powders does not materially change the levels of these vitamins from the levels determined in the fluid whole milks. The requirements of thiamine, niacin, and vitamin B_6 in milligrams per kilogram of ration for pigs (10 to 50 pounds) are reported by the National Research Council (10) to be 1.32, 13.0 to 22.0, and 1.10 mg., respectively. From these calculations, the thiamine and vitamin B_6 levels of the processed milk are marginal for the requirement of the vitamins, whereas the niacin levels of the processed milk are below the requirement as set by the NRC. The level of niacin in the control milk is, also, lower than the requirement levels as reported by NRC (10).

Based upon the results of the chemical analyses and feeding experiments, the ion exchange treatment of milk for removal of cation radionuclides does not seriously affect the nutritive qualities of bovine milk.

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